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ORIGINAL ARTICLE

Human Bocavirus among Viral Causes of Infantile Gastroenteritis

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Key words:

HBoV, Rotavirus, Norovirus, Astrovirus, Adenovirus, Viral G.E

ABSTRACT

Background: *Human bocavirus (HBoV) infection possibly plays a role in gastroenteritis because of the frequent manifestation of gastrointestinal symptoms.* ***Objectives:*** *Detect human bocavirus (HBoV) and assess its prevelance among gastroenteritis associated viral agents in infants with gastroenteritis in Benha University Hospital.* ***Methodology:*** *The study was carried out on 100 stool samples collected from 100 infants with acute gastroenteritis for detection of Rotavirus (RV), Norovirus (NoV) & Astrovirus (AstV) by multiplex reverse transciptase polymerase chain reaction and detection of Adenovirus(AdV) & HBoV by multiplex polymerase chain reaction.* ***Results:*** *Viral agents were detected in 57 (57%) samples; 51 (51%) samples show mono–infection while 6 (6%) samples show co–infection. Rotavirus, Norovirus, Adenovirus and astrovirus were detected in 37%, 14%, 7.0%, and 3% of the study population, respectively; HBoV was detected in 2%.* ***Conclusion:*** *This percentage of HBoV suggests that it might play a minor role in gastroenteritis.*

INTRODUCTION

Acute gastroenteritis is a major cause of childhood morbidity and mortality worldwide. Millions infants die of gastroenteritis related disease or complications annually. Conservative estimates put diarrhea in the top 5 causes of deaths worldwide [1].Viral diarrhea is the most common type of diarrhea in the world affecting patients of all ages. Viruses are recognized as a major cause of gastroenteritis, particularly in children. The number of viral agents associated with diarrheal disease in humans has progressively increased. The most common ones are *Rotaviruses*, *Caliciviruses*, *Norwalk viruses* and *enteric Adenoviruses*. Other viruses such as human *Bocavirus*, *Toroviruses, Coronaviruses*, *Picobirnaviruses* and *Aichi* virus are increasingly being identified as causative agents of diarrhea [2].

Human bocavirus (HBoV) was discovered in 2005 by *Allander et al*. in children with acute respiratory tract infection [3]. Although its clinical epidemiology and role in respiratory infection have not yet been fully elucidated, it was defined as a respiratory pathogen due to the high rate of its detection in respiratory samples [4].

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HBoV was detected in fecal samples of children who had gastroenteritis with or without symptoms of respiratory infection suggesting that HBoV infection possibly plays a role in gastroenteritis and therefore, it could be an enteric pathogen, as well as being a respiratory pathogen [5].

HBoV is a member in the *Parvoviridae* family, *Parvovirinae* subfamily, *Bocavirus genus.* It is further subdivided into four species HBoV 1-4 based on the genomic analysis of the structural (VP1/VP2) and non-structural regions (NS1 and NP1) of the virus [6].

HBoV is small, 20-25 nm in diameter, with minus sense, single stranded DNA genome, non-enveloped with icosahedral capsid symmetry [7]. The aim of this study is detection of human bocavirus (HBoV) and assessment of its prevelance among gastroenteritis associated viral agents in infants with gastroenteritis in Benha University Hospital.

METHODOLOGY

Patients:

This study was carried out on 100 stool samples collected from 100 patients clinically diagnosed as acute gastroenteritis, attending the Pediatric Outpatient and Inpatient Clinics of the Pediatric Department in Benha University Hospital in the period from July 2013 to January 2015. The patients ages ranged from 1 month to 2 years and they were complaining of vomiting, watery diarrhea and abdominal pain. Those with chest

infection, high grade fever, mucous or blood in stool, chronic diarrhea (lasting for more than 2 weeks) were excluded from the study. A written consent was taken from the parents of the selected cases.

Sample collection:

Fecal samples were collected during the first 48hours of symptoms using a sterile cotton swab, transported in viral transport medium (VTM). The sample was vortexed for 15sec, centrifuged for 5min at 14000xg. An InhibitEX tablet (QIAGEN) was added to the supernatant and immediately vortexed for 1min or until the tablet was completely suspended. The suspension was incubated for 1min at room temperature to allow inhibitors to be adsorbed to the InhibitEX matrix. The sample was centrifuged at 14000×g for 3min, the supernatant was collected, transfered into a new 2ml microcentrifuge tube and stored at –70^{o}C.

Viral nucleic acids extraction and purification and Reverse transcription:

Viral nucleic acids were extracted, using the Thermo Scientific GeneJET Viral DNA and RNA Purification Kit and cDNA synthesis was obtained using the FastQuant RT kit with gDNAse (Tiangen Biotech, China)**,** according to the manufacturer's instructions. The extracted DNA and the obtained cDNA wered stored at –20 ^{o}C.

Nucleic acids amplification:

For amplification of the nucleic acids two PCR sets were performed one is a multiplex PCR for amplification of the purified genomic DNA of DNA viruses (Adenovirus & HBoV), the other is a multiplex RT-PCR for amplification of cDNA of RNA viruses (Rotavirus, Norovirus & Astrovirus). The Thermo Scientific Maxima Hot Start Green PCR Master Mix (2X), primers shown in table (1) which targeted to conserved regions of viral genomes, the extracted and the reversly transcriped nucleic acids and nuclease free water were used according to the manufactur instructions.

Table 1: Primers Sequences and amplified product length for PCR and RT-PCR used for detection of the targeted enteric viruses [8].

Virus	Target	Primer	Sequence (5′–3′)	PCR product size (bp)
1. Adenovirus D:\M.D\D. Abeer\PRIME RS.htm - fn-1	VP6	Hex1deg(F)	GCC SCA RTG GKC WTA CAT GCA CAT C	**300**
	VP6	Hex2deg(R)	CAG CAC SCC ICG RAT GTC AAA***	
2. HBoV	VP1/2	Boca-AK-VP-(F)	GGCTCCTGCTCTAGGAAATAAAGAG	**500**
	VP1/2	Boca-AK-VP-(R)	CCTGCTGTTAGGTCGTTGTTGTATGT	
3. Rotavirus	VP7	Rota-Beg-9 (F)	GGCTTTAAAAGAGAGAATTTCCGTCTGG	**1.062**
	VP7	RVG9-(R),	GGTCACATCATACAATTCT	
4. Norovirus	3D	Calici-P290- (F)	GATTACTCCAAGTGGGACTCCAC	**319/331**
	3D	Calici-P289- (R)	TGACAATGTAATCATCACCATA	
5. Astrovirus	3D	Astro-panF11(F)	GARTTYGATTGGRCKCGKTAYGA***	**560**
	3D	Astro-pan-R1(R)	GGYTTKACCCACATICCRAA	

Amplification cycles of multiplex PCR were: Initial denaturation at 95°C for 4min. Followed by repeated cycles of: Denaturation (30sec at 95˚C), annealing (30sec at 50˚C), extension (30sec at 72˚C). Followed by a final extension step at 72°C for15min. Amplification cycles of multiplex RT-PCR were: Initial denaturation at 95°C for 4min. Followed by repeated cycles of: Denaturation (30sec at 95˚C), annealing (30sec at 40˚C), extension (60sec at 72˚C). Followed by a final extension step at 72°C for15min.

Detection of PCR products:

The PCR products were separated by electrophoresis in 1% agarose gel and visualized under UV lamp after ethidium bromide staining [9].

Statistical analysis:

Statistical analysis was performed using SPSS, version 16. $\chi 2$ analysis was used to compare categorical data, Z test was used to compare one outcome in two different groups and Kappa test was used to detect the degree of agreement between different tests. A p value < 0.05 was considered statistically significant.

RESULTS

Out of the 100 patients, sixty (60%) were inpatients and 40 (40%) were outpatients. Fifty one patients (51%) were males and 49 (49%) were females. Sixty one patients (61%) were rural and 39 (39%) were urban. Fifteen patients were (15%) breast fed, 30 (30%) were bottle fed and 55 (55%) were mixed fed. Eighteen (18%) patients aged from1–6 months, 39 (39%) aged from 7–12 months, 27 (27%) aged from 13–18 months and 16 (16%) aged from 19–24 months. The patients were suffering from diarrhea (100%), vomiting (70%),

fever (56%), abdominal pain (52%) and dehydration (42%).

There were 57/100 (57%) samples were positive for viral infection. Rotavirus and Norovirus were the viral agents most frequently detected occurring in 37% and 14% of samples respectively; Adenovirus, Astrovirus and HBoV were detected in 7%, 3% and 2% of samples respectively. Coinfection was found in 6% of samples. In addition to being the viral agents that were most frequent, Rotavirus and Norovirus were also the most frequently co-detected, co-occuring in 2 (2%) of samples, Rotavirus and Adenovirus co- detected in 1(1%) of samples, Rotavirus and Astrovirus in 1(1%), Rotavirus and HBoV in 1(1%), Norovirus and Adenovirus in 1(1%).

Table 2: Distribution of the targeted enteric viruses detected by PCR & RT-PCR in stool samples collected from infants suffering from acute gastroenteritis according to type of the virus:

Type of viruses detected	*No. of viruses detected*	*%*
Rota	37	37
Noro	14	14
Adeno	7	7
Astro	3	3
HBoV	2	2
Total	63	63

The rates of viral infection in inpatients versus outpatients were 30/37 (81.1%) vs 7/37 (18.9%) for Rotavirus, 11/14 (78.6%) vs 3/14 (21.4%) for Norovirus, 5 /7 (71.4%) vs 2/7 (28.6%) for Adenovirus, 2/3 (66.7%) vs 1/3 (33.3%) for Astrovirus while the 2 (100%) HBoV positive samples were detected in inpatients. The infection rate of RV between inpatients and outpatients was statistically significant (p 0.001). However, the infection rate of NoV, AdV, AstV & HBoV between outpatients and inpatients was not statistically significant (p>0.05).

The infection rate of Rotavirus, Norovirus and Astrovirus was higher in males (51.4%, 57.1% & 66.7% respectively) than females (48.6%, 42.9% & 33.3% respectively). While the detection rate of Adenovirus was higher in females (57.1%) and HBoV was equally distributed between the two genders. The infection rate of RV, NoV, AdV, AstV & HBoV between male and female patients was statistically insignificant (p>0.05).

The incidence of Rotavirus in infants aged 1–6 months was 10.8%, 51.4% in infants aged 7–12 months, 32.4% in infants aged 13–18 months and 5.4% in those aged 19–24 months. The incidence of Norovirus was 14.3% in infants aged 1–6 months, 42.9% in infants aged 7–12 months, 28.6% in infants aged 13–18 months and 14.3% in infants aged 19–24 months. The majority of Adenovirus DNA positive samples were detected from infants aged 7–12 months (42.9%) while the majority of samples positive for Astrovirus RNA were detected from infants aged 13–18 months (66.7%). HBoV was detected in infants aged 7–18 months. The infection rate of RV between the different age groups was statistically significant (p 0.05). However, the infection rate of NoV, AdV, AstV & HBoV between the different age groups was statistically insignificant (p>0.05) (Figure1)

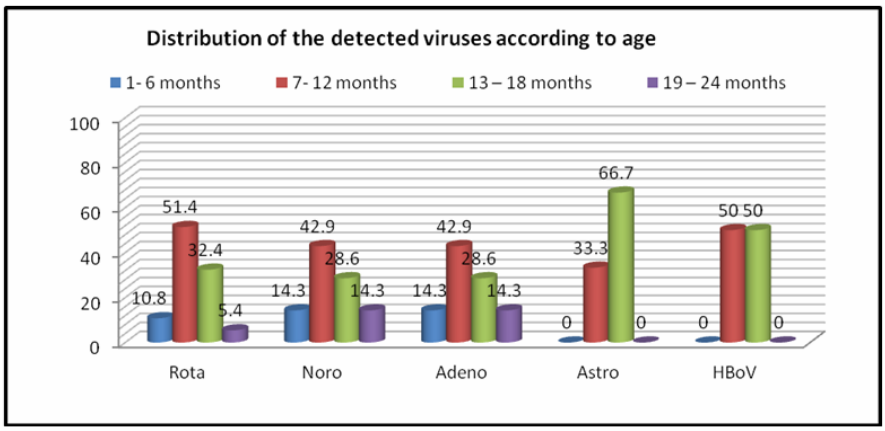

Fig. 1: Distribution of the targeted enteric viruses detected by PCR & RT-PCR in stool samples collected from infants suffering from acute gastroenteritis according to their ages.

Out of Rotavirus positive samples; 13/37 (35.1%) were in bottle fed, 5 /37 (13.5%) were in breast fed and 19/37 (51.3%) were in mixed fed. Out of Norovirus RNA positive samples; 5/14 (35.7%) were in bottle fed, 2/14 (14.3%) were in breast fed and 7/14 (50.0%) were in mixed fed. The majority of Adenovirus & Astrovirus positive samples were detected in mixed fed infants by 57.1% & 66.7% respectively. HBoV was detected only in mixed fed infants. The infection rate of RV & NoV between different patterns of feeding was statistically significant (p 0.001 for RV & <0.05 for NoV). However, the infection rate of AdV, AstV & HBoV between different patterns of feeding was statistically insignificant (p>0.05) (table 2).

Table 3: Distribution of the targeted enteric viruses detected by PCR & RT-PCR in stool samples collected from infants with acute gastroenteritis according to the nutritional state:

Gr. / PCR	*Bottle feeding*		*Breast feeding*		*Mixed feeding*		*Total*		*p-value*
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	
Rota	13	35.1	5	13.5	19	51.3	37	100	< 0.001
Noro	5	35.7	2	14.3	7	50.0	14	100	< 0.05
Adeno	2	28.6	1	14.3	4	57.1	7	100	> 0.05
Astro	1	33.3	0	0	2	66.7	3	100	> 0.05
HBoV	0	0	0	0	2	100	2	100	> 0.05

The rate of viral infection was higher in rural patients than urban patients. It was 24/37 (64.9%) for Rotavirus, 9/14 (64.3%) for Norovirus, 4/7 (57.1%) for Adenovirus, 2/3 (66.7%) for Astrovirus and 100% for HBoV. The rate of infection of RV, NoV, AdV, AstV & HBoV between rural and urban patients was statistically insignificant ($p > 0.05$).

Out of the 37 (100%) Rotavirus infected infants; 37 (100%) infants were suffering from diarrhea, 28 (75.7%) were complaining of vomiting, 23 (62.2%) were have abdominal pain, 21 (56.8%) infants were have fever, 15 (40.5%) were mild dehydrated, 11(29.7%) were moderate dehydrated and 4 (10.8%) infants were sever dehydrated. Out of the 14 (100%) infants infected with Norovirus; 14 (100%) were complaining from diarrhea, 10 (71.4%) infants were complaining from vomiting, 8 (57.1%) were have abdominal pain, 5 (35.7%) infants were have fever, 5 (35.7%) were mild dehydrated, 2 (14.3%) infants were moderate dehydrated and1 (7.1%) was sever dehydrated. Out of the 7 (100%) Adenovirus infected infants; 7 (100%) infants were suffering from diarrhea, 3 (42.9%) were complaining of vomiting, 1(14.3%) infant was had abdominal pain, 2 (28.6%) were had fever and 3 infants (42.9%) were mild dehydrated. Out of the 3 (100%) Astrovirus infected infants; 3 (100%) infants were have diarrhea, 2 (66.7%) were have vomiting, 1 (33.3%) infant was have abdominal pain. Out of the 2 (100%) HBoV infected infants; 2 (100%) infants were have diarrhea, 1 (50%) infant was have vomiting, 1 (50%) infant was have abdominal pain, 1 (50%) was mild dehydrated.

DISCUSSION

Acute gastroenteritis is a common disease among infants and children worldwide. Its associated dehydration is a major cause of morbidity and mortality in pediatric populations. Enteric viruses have been recognized as the most significant etiological agents of acute gastroenteritis in children less than 5 years of age[10].

In the present study, viral agents were identified in 57/100 (57%) cases. Rotavirus and norovirus were the most frequently detected agents followed by a low frequency of adenovirus, astrovirus and HBoV. The overall prevalence of Rotavirus was 37%, Norovirus was 14%, Adenovirus was 7%, Astrovirus was 3% and HBoV was 2%. Co-infection was found with a high incidence between Rotavirus and Norovirus (2%) while it was 1% between Rotavirus and Adenovirus, 1% between Rotavirus and Astrovirus, 1% between Rotavirus and HBoV and 1% between Norovirus and Adenovirus.

Albuquerque et al.[11] in Brazil, found that a potential pathogen was found in 30.5% of samples, 2% of diarrhea stool samples were positive for HBoV by PCR. RV was detected in 11.9% of samples, AdV was detected in 4.8% of samples, NoV was found in 3.4% of samples and AstV was present in 0.3% of samples. Co-infection with other enteric viruses was found in 21.4% of HBoV positive samples. In agreement with a study carried out in Korea, by *Lee et al.* [12] who reported that viral agents were found in 44.4% of the study population; RV and NoV were the most frequently detected, occurring in 25.7% and 13.7% of the study population, respectively; AdV, AstV and HBoV were detected in 3.0%, 1.1% and 0.8% of the study population, respectively. A single viral agent was detected in 39.7% while mixed infection was found in 2.4% of the study population. In addition to being the viral agents that were most frequent, RV and NoV also were those most frequently co-detected, co-occurring in 1.1% of the study population; NoV and AdV were co-detected in 0.5% , RV and HBoV in 0.3%, AstV and HBoV in 0.1% , NoV and HBoV in 0.1%, RV and AstV in 0.1% and NoV and AstV in 0.1%. These great variations in the frequencies of enteric pathogens identified in different epidemiologic studies are dependent on several parameters such as country and type of method used for diagnosis[13].

In our study viral incidence was higher in inpatients (75.4%) than in outpatients (24.6%). 81.1% versus 18.9% for Rotavirus, 78.6% versus 21.4% for Norovirus,71.4% versus 28.6% for Adenovirus, 66.7% versus 33.3% for Astrovirus while 100% of HBoV were detected in inpatients. The infection rate of RV between inpatients and outpatients was statistically significant ($p<0.001$). However, the infection rate of NoV, AdV, AstV & HBoV between outpatients and inpatients was not statistically significant ($p>0.05$).

Sai et al. [14] in a similar study in China, found that rates of RV and NoV among outpatients were 30.5% and 11.6% respectively while they were 41.5% and 8.3% for RV and NoV respectively among inpatients. The infection rate of Rotavirus between inpatients and outpatients was statistically significant (P = 0.002). However, the infection rate of Norovirus between outpatients and inpatients was not statistically significant (P = 0.161). *Albuquerque et al.*[11] performed a study on children with gastroenteritis in Brazil, and found that the burden of HBoV was 71.4% in hospitalized children while it was28.7% in outpatients.

In the present study, viral incidence was higher in males 54.4% than females 45.6%.The incidence was 51.4% males and 48.6% females for Rotavirus, 57.1% males and 42.9% females for Norovirus, 42.9% males and 57.1% females for Adenovirus, 66.7% males and 33.3% females for Astrovirus, 50% for males and females concerning HBoV. Although the difference in the infection rate of RV, NoV, AdV, AstV & HBoV between male and female patients it was statistically insignificant (p>0.05).

In a study conducted in Ghana, *Chen et al.* [15] found that the proportion of RV positive specimens was higher in boys (50.6%) than in girls (37.1%) in contrast to the proportion of NoV positive samples which was higher in girls (17.7%) than in boys (11.7%). On the other hand, *Wu et al.* [16] in Taiwan, reported that the incidence of RV was 48.1% in males and 51.9% in females while the incidence of NoV was 63.6% in males and 36.4% in females. *Rezaei et al.* [17] performed a study in Iran, they found that about 62.5% of positive cases for AdV infection were in girls and 37.5% were in boys with no statistical significance (p>0.05%). In contrast to another study in Iran, conducted by *Zadeh et al.*[18] reported that AdV positive cases were 58.3% in males and 41.7% in females. In UK, *Nawaz et al.* [19] reported that the distribution of HBoV among females and males was not significantly different from the distribution of females and males in the entire cohort which was 53% and 47%, respectively. *Albuquerque et al.* [11] carried out a study on HBoV in Brazil, found that 57% of HBoV positive cases were detected in boys and 43% were detected in girls.

In our study, viral infections show the highest incidence (43.9%) in infants aged 7–12 months. The incidence of Rotavirus, Norovirus and Adenovirus was high among infants of this age group by 45.9%, 42.9% and 42.9% respectively. While the incidence of Astrovirus increased among infants aged 13–18 months, HBoV was equally distributed in patients aged 6–18 months. The infection rate of RV between the different age groups was statistically significant (p<0.05). However, the infection rate of NoV, AdV, AstV & HBoV between the different age groups did not reach a significant level (p>0.05).

Another Egyptian study carried out by *El-Mohammady et al.* [20] who reported that RV, NoV, AdV and AstV mostly occur in the 0–12 months age group. In a study conducted in China, *Cheng et al.* [21] reported that the majority of patients who were positive for RV, AstV and AdV were 0–12 months of age, whereas NoVs and HBoV were found mostly in children aged 7–18 months. The peak age incidence for virus gastroenteritis is between 6–18 months of age, it is less frequent in the first 6 months of life and this may be due to exclusive or partial breast feeding in the first 6 months. As trans-placental acquired immunity may prevent infection in the first months of life. The lower incidence of virus gastroenteritis in children over 18 months of age might be due to acquired immunity from previous infections [22].

In the present study, the incidence of viral infection was highest (52.6%) among mixed fed infants. The incidence was 51.3% Rotavirus, 50.0% Norovirus, 57.1% Adenovirus, 66.7% Astrovirus and 100% HBoV. The infection rate of RV & NoV between different patterns of feeding was statistically significant (p 0.001 for RV & 0.05 for NoV). However, the infection rate of AdV, AstV & HBoV between different patterns of feeding was statistically insignificant (p > 0.05).

Al-Dahmoshi et al. [23] in Iraq, found that viral diarrheal infections among mixed fed infants were three time (76.7%) lager than those who were breast fed (23.3%). With the prevalence of RV, NoV and AdV was 80.8%, 63.3% and 85.7% respectively among mixed fed and 19.2%, 36.7%, 14.3% respectively among breast fed infants. The low incidence of infection among breast fed infants is because human milk contains human viral specific antibodies which are capable of neutralizing viral antigens [22].

In our study the incidence of viral infection was higher in rural patients (64.9%) than urban patients (35.1%). The incidence was 64.9% versus 35.1% for Rotavirus, 64.3% versus35.7% for Norovirus, 57.1% versus 42.9 for adenovirus, 66.7% versus 33.3% for Astrovirus and 100% rural for HBoV. The rate of infection of RV, NoV, AdV, AstV & HBoV between rural and urban patients was statistically insignificant (p>0.05).

This is in agreement with a study carried out in Iraq, by *Al-Dahmoshi et al.*[23] who found that the incidence of viral diarrhea caused by RV, NoV and AdV was high among rural infants (69.2%, 60% and 85.7%) than those in urban infants (30.8%, 40%, 14.3%).

In our study, it was found that the clinical picture was more prominant with rotavirus infection in comparison with other viruses as all (100%) rotavirus infected infants had diarrhea, 75.7% had vomiting, 62.2% had abdominal pain, 56.8% had fever, 40.5% had mild dehydration, 29.7% had moderate dehydration and10.8% had severe dehydration.

In a study carried out in Sudan, *Magzoub et al.* [24] reported that 98.3% of RV infected children, had

diarrhea, 82.6% had vomiting, 58.7% had fever,71.1% had severe dehydration, 28.1% had mild dehydration while 0.8% without signs of dehydration. *Sherchand et al.* [25] in a similar work in Nepal, found that all (100%) RV positive children had diarrhea, 27.9% had Vomiting, 23.9% had abdominal pain, 23.4% had fever, 13.02% had mild dehydration, 25.8% had moderate dehydration and 22.9% had severe dehydration. In China, *Sai et al.*[14] reported that the major clinical symptoms related to NoV gastroenteritis were acute diarrhea, vomiting and fever with an incidence of 13.8% for diarrhea, 67.5% for vomiting and 46.3% for fever. On the other hand, *Ahmed et al*. [26] in Turkey, reported that out of NoV positive cases, 78% had watery diarrhea and 67% experienced vomiting. *Jarecki et al.* [27] found that the most common presentation of AdV infection was diarrhea (87.5%), vomiting (80.0%), abdominal pain (76.3%) and low grade fever (95.0%). In a study conducted in Argentina, by *Giordano et al.*[28] on astrovirus associated diarrhea they reported that all (100%) AstV positive cases had diarrhea, 41.7% had fever, 25.0% had vomiting and 8.3% had dehydration. *Albuquerque et al.*[11] in Brazil, found that all HBoV infected patients had diarrhea (100%), 14.3% had fever and7.2% had vomiting. In a similar work *Lee et al.*[12] reported that all HBoV positive patients had diarrhea,62.5% had fever and 37.5% had vomiting**.**

CONCLUSION

The study reveals that viral gastroenteritis could be diagnosed by conventional polymerase chain reaction as a rapid and sensitive technique. Rotavirus and Norovirus were the most frequent agents and the most common co-infectious agents responsible for gastroenteritis , HBoV was detected in 2% of the selected cases. Detection of human bocavirus (HBoV) in stool of children with gastroenteritis without symptoms of respiratory infection suggesting that HBoV infection possibly plays a role in gastroenteritis.

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